

**UNCLASSIFIED**

**AD 453400**

**DEFENSE DOCUMENTATION CENTER**

**FOR**

**SCIENTIFIC AND TECHNICAL INFORMATION**

**CAMERON STATION ALEXANDRIA, VIRGINIA**



**UNCLASSIFIED**

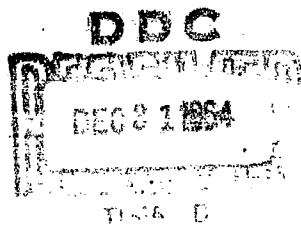
NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

4 5 3 4 0 0  
CATALOGED BY DDCI  
AD No. 453400  
AS

TECHNICAL MANUSCRIPT 161

DIAGNOSIS OF TULAREMIA BY  
FLUORESCENT-ANTIBODY TECHNIQUES

SEPTEMBER 1964



UNITED STATES ARMY  
BIOLOGICAL LABORATORIES  
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES  
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 161

DIAGNOSIS OF TULAREMIA BY FLUORESCENT-ANTIBODY  
TECHNIQUES

John D. White

Malcolm H. McGavran

Pathology Division  
DIRECTOR OF MEDICAL RESEARCH

Project 1C622401A072

September 1964

This publication or any portion thereof may not be reproduced without specific authorization from the Commanding Officer, U. S. Army Biological Laboratories, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland. 21701. However, DDC is authorized to reproduce the publication for U. S. Government purposes.

The information in this publication has not been cleared for release to the public.

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this publication directly from LDC.

Foreign announcement and dissemination of this publication by DDC is limited.

ABSTRACT

P. tularensis, the causative organism of tularemia, can be readily and positively identified in formalin-fixed and paraffin-embedded human tissues. This was done in eight of nine cases examined. The diagnostic and therapeutic implications of this advance are discussed.

## I. INTRODUCTION

Tularemia is a sporadic disease in man. Its diagnosis may be difficult, particularly when the disease manifests itself in an atypical form or has been modified by antibiotic therapy. The most satisfactory method for positive diagnosis is bacteriologic culture. Pasteurella tularensis is cytotropic with fastidious growth requirements and, thus, special media and some degree of microbiological skill are needed to ensure recovery of the organisms in culture. In addition, there is a definite danger of infection for the personnel in the laboratory. Therefore, an accurate and rapid method for the identification of P. tularensis that is not dependent upon the culture of viable organisms would be of considerable value.

The technique for identifying antigens by fluorescent-tagged antibodies was introduced by Coons and Kaplan,<sup>1</sup> and after some technical modifications has been used in many areas of research and clinical application. The reviews of Coons,<sup>2</sup> Cherry and co-workers,<sup>3</sup> Beutner,<sup>4</sup> and Smith<sup>5</sup> summarize the applied and theoretical status of fluorescent antibody techniques.

The purpose of this paper is to demonstrate the practical application of fluorescein-labeled antibodies for the detection of P. tularensis in human tissues that have been fixed in formalin and embedded in paraffin.

## II. MATERIALS AND METHODS

The necropsy records of the Department of Pathology, Washington University, were examined and seven cases of tularemia necropsied during the period 1937 to 1941 were found. The clinical and pathological protocols were reviewed and appropriate paraffin blocks and wet, fixed tissues were used to make new histologic sections. The wet tissues had been either fixed and stored in 10 per cent formalin or fixed in Zenkers-formol (Helley's) and stored in 70 per cent ethanol. Case 8 was necropsied in 1960. In addition, a surgically excised lymph node from the axilla of a woman with ulceroglandular tularemia (Case 9) was studied.

The new histologic sections were stained with hematoxylin and eosin, Giemsa, and Gram's stains. Two additional sections of each tissue were used for the detection of organisms by fluorescent antibodies. These methods are described elsewhere.<sup>6,7</sup>

### III. RESULTS

The results are summarized in Table I. With one exception, P. tularensis was identified in at least one tissue from each case. Six of these nine persons had some contact with rabbits prior to illness. Five developed the ulceroglandular form of the disease and four had typhoidal tularemia. In Cases 1 through 8, which were fatal, pneumonic involvement was demonstrated at necropsy. P. tularensis was cultured in only one case and isolated by inoculation of guinea pigs in three instances. In four cases, specific agglutinin titers for P. tularensis were elevated significantly.

It is apparent that the diagnosis of tularemia in six of the nine patients was based on clinical, serologic, or morphologic grounds. The morphologic appearance of the lesions in tularemia is related to the stage of the illness. Early in the disease, focal necrosis is evident. Granulomatous lesions are characteristic in later stages of tularemia. In the pre-antibiotic era the morphologic appearance of tularemia was most usually of the granulomatous type. However, in cases that have been treated with antibiotics, clinical features and pathological anatomy may be modified. Case 8 illustrates this point in which the lesions were atypically granulomatous (Figure 1).

It is generally accepted that P. tularensis cannot be demonstrated in the usual histologic preparations of human tissues. In neither the Giemsa- or Gram-stained sections of these cases were microorganisms of appropriate morphology found. However, using fluorescent antibodies, coccobacillary microorganisms with specific immunochemical reactivity of P. tularensis were identified in many of these tissues (Table I and Figure 2). Tissues fixed in either Zenker's formalin and stored in 70 per cent ethanol or fixed and stored in 10 per cent formalin were satisfactory. The blue auto-fluorescence of the Zenker-fixed tissue was bright and in many instances made it difficult to get satisfactory photomicrographs. It did not, however, mask the specific yellow-green fluorescence of the conjugated antibody.

### IV. DISCUSSION

The retention of antigenic reactivity of P. tularensis after prolonged storage in formalin or alcohol and subsequent processing into paraffin was not altogether unsuspected. Foshay's initial vaccine was prepared from formalin-treated cells of P. tularensis.<sup>8</sup> It was antigenic.

TABLE I. RESULTS OF FLUORESCENT ANTIBODY STUDIES ON NINE CASES OF HUMAN TULAREMIA

Case #/ Age, Sex,	Year	Age/Sex Rabbits	Contact With Tularemia	Type of Tissue	Culture/ Animal Indic.	Elevation of Serum Agglutin- ing Titers		Fluorescent Antibody Ident. of <i>B. tularensis</i>	
						Lung	Lymph Node	Spleen	Gut
1/891	1937	3/F	-	Typhoidal	+/-	-	N.A.	+	+
2/825	52/M	4/2/M	+	Visceroglandular	-/-	+	N.A.	+	N.A. +
3/827	57/M	-	-	Typhoidal	-/-	-	N.A.	N.A.	N.A.
4/844	62/M	52/M	+	Visceroglandular	-/-	-	N.A.	N.A.	-
5/845	76/F	75/F	+	Visceroglandular	-/-	-	N.A.	N.A.	-
6/8957	29/2	45/F	+	Typhoidal	-/-	+	+	+	-
7/842	24/M	43/M	-	Typhoidal	-/-	+	+	+	N.A. N.A.
8/821	47	48/M	+	Visceroglandular	-/-	-	+	N.A.	N.A. N.A.
9/8425	17	17/F	+	Visceroglandular	-/-	+	+	+	-

(+) = tissue not available.  
 (-) = absent of axillary lymph node.

The results obtained in the present study appear to establish the practicability of using fluorescent antibodies for the identification of P. tularensis in human tissues. Positive identification was not made in one case (No. 3). This material was stored for 25 years but the lungs were the only tissue available for examination. It should be noted that there was no history of contact with rabbits, P. tularensis was not isolated, and serum agglutinins were not elevated.

As more and more diagnostic laboratories begin to use fluorescent antibody techniques, it would be desirable to include conjugated antisera for P. tularensis. In order to start streptomycin therapy promptly, tularemia could be expeditiously and safely diagnosed using smears or biopsies fixed in formalin. Other applications could be those in the study of granulomatous inflammatory processes of uncertain etiology in either surgically excised specimens or material from necropsy.

LITERATURE CITED

1. Coons, A.H. and Kaplan, M.H. "Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody," *J. Exptl. Med.* 91:1-13, 1950.
2. Coons, A.H. "Fluorescent Antibody Methods," In: Danillli, J.F., ed. "General Cytochemical Methods," Vol. I, Academic Press, Inc., New York, 1958.
3. Cherry, W.B.; Goldman, M.; Carshi, T.R.; and Moody, M.D. "Fluorescent antibody techniques," Superintendent of Documents, U.S. Government Printing Office, Washington 25, D.C. (U.S. Public Health Service Publication 729, 1960)
4. Beutner, E.H. "Immunofluorescent staining: The fluorescent antibody method," *Bacteriol. Rev.* 25:49-76, 1961.
5. Smith, C.W.; Metzger, J.F.; and Heggan, M.D. "Immunofluorescence as applied to pathology," *Am. J. Clin. Pathol.* 38:26-42, 1962.
6. McGavran, M.H.; White, J.D.; Eigelsbach, H.T.; and Kerpsack, R.W. "Morphologic and immunohistochemical studies of the pathogenesis of infection and antibody formation subsequent to vaccination of Macacus irus with an attenuated strain of Pasteurella tularensis: I. Intracutaneous vaccination," *Am. J. Pathol.* 41:405-413, 1962.
7. White, J.D.; Rooney, J.R.; Prickett, P.A.; Derrenbacher, E.B.; Beard, C.W.; and Griffith, W.R. "Experimental respiratory tularemia," *J. Infect. Diseases* 114:277-283, 1964.
8. Foshay, L. "Prophylactic vaccination against tularemia," *Am. J. Clin. Pathol.* 2:7-10, 1932.